Serine integrase chimeras with activity in E. coli and HeLa cells.

Journal: Biol Open

Publication Year: 2014

Authors: Alfonso P Farruggio, Michele P Calos

PubMed link: 25217617

Funding Grants: Safe, efficient creation of human induced pluripotent stem cells without the use of retroviruses, Stem Cell Therapy for Duchenne Muscular Dystrophy, Site-specific integration of Lmx1a, FoxA2, & Otx2 to optimize dopaminergic differentiation, Engineered iPSC for therapy of limb girdle muscular dystrophy type 2B

Public Summary:
The Calos lab has been a leader in developing serine phage integrases for use in genetic engineering of human cells. These types of enzymes come from the viruses of certain bacteria, yet they work well in mammalian cells. This class of enzymes, the serene integrates, are able to recombine two DNA sequences. That is, they can cut two different sequences and rejoin them so that they are now linked in a new combination. This type of reaction is useful for integrating foreign DNA sequences into the human genome. For example, if one wants to add a beneficial gene to the chromosomes of human cells, integrases can be used to carry out that reaction. In the past, we showed that some enzymes in this family can recognize existing DNA sequences in human cells, while others can only recognize a DNA sequence found in bacteria that does not exist in human cells. It would be helpful for research if we could design enzymes to recognize specific DNA sequences and perform recombination only at those sequences. That was a goal we had in mind when we performed the research in this study. We know that thousands of different phage integrases exist in nature, each recognizing a different DNA sequence. Some of these sequences would be of interest for placing genes into the human genome. However, even if the DNA recognition part of the enzyme was of interest, the rest of the enzyme, the part, or "domain". that carries out the cutting and pasting reaction, might not work in human cells. To address this problem, we connected a working reaction domain with a DNA recognition domain. We tried out several combination in this study, using pieces from three different integrases. We were able to find hybrids or "chimeras" that work in bacteria and in some cases, human cells. These hybrid enzymes may be useful to create new tools for manipulating DNA. For example, in our studies, we need to repair mutations and add new genes to stem cells in order to create healthy stem cells from patients with muscular dystrophy. The concepts we explored in this study may help us develop new tools for altering stem cells in a safe and predictable manner.

Scientific Abstract:
In recent years, application of serine integrases for genomic engineering has increased in popularity. The factor-independence and unidirectionality of these large serine recombinases makes them well suited for reactions such as site-directed vector integration and cassette exchange in a wide variety of organisms. In order to generate information that might be useful for altering the specificity of serine integrases and to improve their efficiency, we tested a hybridization strategy that has been successful with several small serine recombinases. We created chimeras derived from three characterized members of the serine integrase family, phiC31, phiBT1, and TG1 integrases, by joining their amino- and carboxy-terminal portions. We found that several phiBT1-phiC31 (BC) and phiC31-TG1 (CT) hybrid integrases are active in E. coli. BC chimeras function on native att-sites and on att-sites that are hybrids between those of the two donor enzymes, while CT chimeras only act on the latter att-sites. A BC hybrid, BC[-1], was also active in human HeLa cells. Our work is the first to demonstrate chimeric serine integrase activity. This analysis sheds light on integrase structure and function, and establishes a potentially tractable means to probe the specificity of the thousands of putative large serine recombinases that have been revealed by bioinformatics studies.